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Shedding B Cell Identity

In this issue of *Immunity*, Delogu et al. (2006) and Nera et al. (2006) find that the downregulation of Pax-5 during antigen-dependent-terminal differentiation results in a substantial loss of B cell identity and the transition to a plasma cell state.

The transcription factor Pax-5 functions downstream of the regulators E2A and EBF to reinforce B cell fate choice by activating the expression of B lineage genes such as mb-1, CD19, and BLNK (Busslinger, 2004; Singh et al., 2005). Pax-5 also plays a crucial role in B cell fate commitment by repressing the expression of alternate lineage genes such as c-fms and Notch-1. Pax-$5^{-/-}$ pro-B cells, unlike their wild-type counterparts, are multipotent and can generate T, NK, and myeloid cell types. Finally, conditional deletion of Pax-5 in committed pro-B cells also results in the reversion to a multipotent state from which T lymphocytes and macrophages can be generated (Mikkola et al., 2002). This result suggests that Pax-5 is continuously required to maintain B cell identity. These analyses raise several important issues. How large is the set of lineage-inappropriate genes that are repressed by Pax-5? Are these genes actively repressed by Pax-5, not only in committed pro B cells but also in mature B cells? Finally, because the terminal differentiation of B cells into plasma cells involves a significant loss of B cell identity and is accompanied by the downregulation of Pax-5, could Pax-5 also function to repress the plasma cell gene expression program? Two articles in this issue of *Immunity* experimentally address these questions by examining the consequences of deleting the Pax-5 gene in murine pro-B and mature B cells and the chicken DT40 B cell line.

Busslinger and colleagues comprehensively analyze Pax-5-repressed genes in pro-B cells by comparing the gene-expression profiles of wild-type and Pax-$5^{-/-}$ pro-B cells by using a customized microarray. This analysis reveals a diverse set of 110 genes, many of which are normally expressed in alternate lineages of the hematopoietic system. The observation that a significant number of myeloid genes are misexpressed in Pax-$5^{-/-}$ pro-B cells prompted the authors to analyze the expression pattern of myeloid genes in common lymphoid progenitors (CLPs), which do not express significant levels of Pax-5 and had previously been reported to exhibit a lymphoid-restricted gene expression pattern (Miyamoto et al., 2002). Delogu et al. (2006) observe that CLPs like Pax-$5^{-/-}$ pro-B cells indeed express detectable myeloid gene transcripts and suggest that this accounts for their latent myeloid potential. Thus, CLPs can be viewed as early B lineage developmental intermediates in the bone marrow that are in the process of undergoing restriction in their myeloid versus B lineage developmental potential.

To determine if Pax-5 is continuously required for repression of alternate lineage genes, the authors examine the consequences of Pax-5 loss in lymph node B cells, using a conditional deletion strategy (CD19-cre). This shows that alternate lineage genes such as *Ccl3*, *Csf1r (M-CSFR)*, and *Fcεr1γ* require Pax-5 for their repression in pro-B as well as B cells. Intriguingly, loss of Pax-5 in B cells also results in the activation of genes that are required for and/or associated with plasma cell differentiation, such as *Prdm1 (Blimp-1)*, *Igj (J chain)*, *Cd28*, and *Ccr2*. Blimp-1 is an essential transcriptional regulator of plasma cell differentiation (Shapiro-Shelef and Calame, 2005). Because Pax-5 expression is downregulated during antigen-dependent terminal differentiation of mature B cells into immunoglobulin-secreting plasma cells, this leads to the possibility that modulation of Pax-5 expression is an obligate event that controls the B to plasma cell transition (Figure 1).

Does the continuous repression of lineage inappropriate and plasma cell genes by Pax-5 in pro-B and B cells have functional consequences? The authors nicely test both possibilities. Using a transgenic strategy, they misexpress the Pax-5-repressed chemokine gene *Ccl3* throughout B cell development. This results in enhanced generation of osteoclasts and bone loss, suggesting that Pax-5-dependent gene repression is required for homeostasis within the hematopoietic system. Conversely, the Pax-5-repressed genes *Cd28* and *Ccr2*, which are expressed in plasma cells, are shown to have cell intrinsic functions in postgerminal center B cells. Thus, the continuous function of Pax-5 in actively repressing genes during B cell development may have evolved from the requirement to shed B cell identity during terminal differentiation into plasma cells.

Nera et al. (2006) use the chicken DT40 B cell line to analyze the functions of Pax-5. Deletion of both Pax-5 alleles results in reduced surface IgM expression and loss of B cell receptor signaling. This is attributed to downregulation of genes encoding Ig coreceptor and signaling molecules such as Igβ, BLNK, and Lyn. Importantly in Pax-$5^{-/-}$ DT40 B cells, the plasma cell transcription factors Blimp-1 and XBP-1 are induced, whereas the germinal center transcriptional regulator Bcl-6 is downregulated. Consistent with these changes in the transcriptional regulatory network, the mutant DT40 cells secrete elevated levels of IgM. Thus, in this cell system, deletion of Pax-5 results both in a loss of B cell identity and a substantial transition to a plasma cell state. The latter is likely due to loss of Bcl-6 expression, as reexpression of Bcl-6 in Pax-$5^{-/-}$ B cells blocks the induction of Blimp-1. These results lead the authors to suggest that Pax-5 positively regulates B cell identity as well as the germinal center B cell program, the latter by sustaining the expression of Bcl-6. In this regard, Pax-5 also appears to positively regulate AID expression. After affinity maturation and isotype switching, Pax-5 is downregulated, resulting in diminished expression of Bcl-6, thereby enabling the induction of plasma cell transcription factors Blimp-1 and XBP-1. In this regulatory model, Pax-5 represses Blimp-1 indirectly via Bcl-6 (Figure 1).

Although these experiments analyzing Pax-5 function in murine B cells and the avian DT40 cell line reinforce

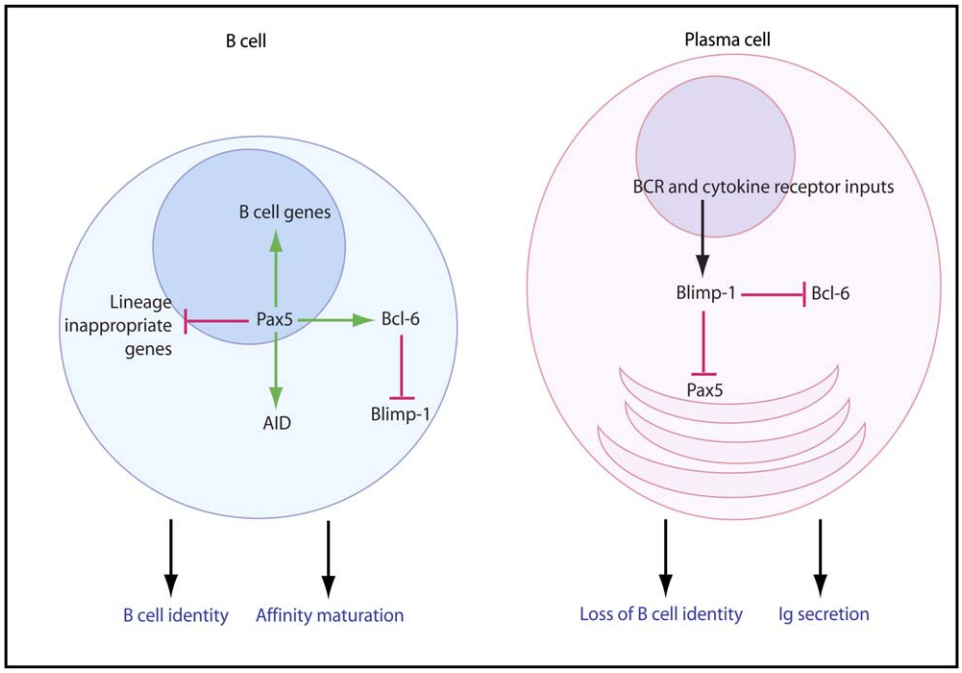

Figure 1. A Model that Illustrates the Role of Pax5 in Maintaining B Cell Identity and in Repressing Plasma Cell Differentiation

During the antigen-independent phases of B cell differentiation, Pax5 sustains B cell identity by simultaneously activating genes necessary for B cell function and repressing a large cohort of lineage inappropriate genes. After antigenic stimulation, Pax5 continues its roles in maintaining B cell identity and upregulates the expression of Bcl-6 and AID, genes necessary for affinity maturation. Importantly, Pax5-induced expression of Bcl-6 serves to repress the expression of Blimp-1 and, ultimately, plasma cell differentiation. In a plasma cell, Pax5 expression is turned off, in part by Blimp-1, which leads to the loss of B cell identity. In addition, Blimp-1 represses the expression of Bcl-6, thus cementing the plasma cell state necessary for efficient antibody secretion. Thus, the expression of Pax5 in B cells serves to preserve B cell functions necessary for efficient immunity, whereas its dynamic downregulation during the conversion to a plasma cell allows Blimp-1 expression that results in a terminally differentiated, antibody-secreting plasma cell.

each other, they differ from the standpoint that loss of Pax-5 in the latter system results in a more profound transition to a plasma cell state. This is likely due to the fact that although loss of Pax-5 in murine B cells results in enhanced levels of Blimp-1, it is not accompanied by reduced expression of Bcl-6 or elevated levels of XBP-1. The differences between the two systems may reflect a species variation in the regulatory circuitry or, alternatively, the fact that the DT40 cells reflect an activated germinal center B cell state. According to the latter view, the induction of physiological levels of Blimp-1 expression may depend on both the downregulation of Pax-5 as well as positive regulatory inputs from the antigen and cytokine receptor signaling systems. Thus, loss of Pax-5 at this later activated stage of B cell development may account for the fuller transition to a plasma cell state.

Nevertheless, both experimental systems demonstrate that Pax-5 is continuously required for maintaining B cell identity and repressing the plasma cell program. Its downregulation at a late stage of antigen-driven B cell differentiation results in loss of B cell identity and the emergence of the plasma cell state. Thus, Pax-5 downregulation in addition to Bcl-6 degradation (Niu et al., 1998) likely trigger the onset of plasma cell differentiation, and it will be important to explore the underlying regulatory pathways and molecular mechanisms.

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